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Evaluation of neutrophil lymphocyte ratio as a venous risk factor in patients with primary familial erythrocytosis

Alpay Yeşilaltay
Department of Hematology, Faculty of Medicine, Baskent University, Istanbul Research Hospital, Istanbul, Turkey

Abstract

Aim: Primary Familial Erythrocytosis (PFE) is an inherited disorder characterized by polycythemia. Studies have shown that these patients are frequently predisposed to atherosclerosis and thromboembolic complications. In our study, we aimed to analyze the risk of atherosclerosis in PFE patients using the Neutrophil-to-Lymphocyte Ratio (NLR), which is considered a potential predictive factor for atherosclerosis in recent years.

Material and Methods: A total of 50 patients, including 2 females and 48 males diagnosed with polycythemia, were enrolled in the study. All patients tested negative for JAK2 V617F and Exon 12 mutations, and their EPO levels were within the normal range, leading to the diagnosis of PFE. Patients with high EPO levels and/or positive JAK analyses, those with any history of cancer, those undergoing active chemotherapy, and those with cardiac and respiratory diseases were excluded from the study. Hemogram evaluations of all patients were conducted in the absence of active infection that could affect neutrophil and lymphocyte counts. The ratio of neutrophil counts to lymphocyte counts in patients was evaluated and analyzed.

Results: The NLR threshold was accepted as 2.3 for increased risk of atherosclerosis. NLR > 2.3 was observed in 94% of the patients included in the study (except for 3 patients). The mean NLR value for all patients was found to be 2.86.

Discussion: NLR has been interpreted as an early indicator of atherosclerosis, particularly in recent studies on cardiovascular diseases. It is known that PFE predisposes individuals to atherosclerosis, thromboembolism, and cardiovascular diseases. Considering the early onset of PFE and its genetic transmission, we believe that NLR could serve as an early determinant parameter for atherosclerosis in this disease.

Keywords

Neutrophil, Lymphocyte, Atherosclerosis, Erythrocytosis

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Corresponding Author: Alpay Yeşilaltay, Department of Hematology, Faculty of Medicine, Baskent University, Istanbul Research Hospital, 34662, Istanbul, Turkey.
E-mail: alpay.yesilaltay@hotmail.com P: +90 507 240 42 84
Corresponding Author ORCID ID: https://orcid.org/0000-0003-3784-5739
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Introduction

The most important parameter that regulates erythropoietin secretion under normal conditions in the body is the haemoglobin level in erythrocytes. Under hypoxic conditions, HIF-1 (hypoxic inducible factor-1) is the major transcription gene responsible for the transcription of the EPO gene. This factor is hydroxylated and bound to VHL protein with the help of PHD protein in normoxaemic states. Afterwards, this bound structure is removed from the environment as a degree in the proteasome system. Furthermore, under hypoxaemic conditions, HIF-1 alpha binds to the EPO gene together with HIF-1 beta, leading to EPO formation and consequently an increase in erythrocyte levels [1].

Erythrocytosis is defined by an increase in haematocrit above the upper limit of normal. According to the latest WHO 2016 criteria, polycythemia is accepted as Hb 16.5 g/dL in males and above 16 g/dL in females, and haematocrit is accepted as 49% in males and 48% in females [2].

Erythrocytoses occur clinically as congenital or acquired and are classified as primary (autonomous, due to bone marrow progenitor cell defects) or secondary (due to increased EPO or other erythropoietic factors). Primary erythrocytoses characterised by low or subnormal EPO levels are divided into two groups: hereditary (EPO receptor mutations) and acquired (polycythemia vera), whereas secondary erythrocytoses characterised by high EPO levels may occur due to hereditary causes, low tissue oxygenation, some tumours, renal diseases, adrenal cortical hyperfunction and androgen therapy [3].

Primary familial erythrocytosis (PFE) occurs as a result of isolated and abnormal proliferation of erythroid cells in the bone marrow. This disease is typically inherited in an autosomal dominant pattern, Clinically isolated erythrocytosis, normal leukocyte and platelet counts, low serum EPO level (<10mU/ml), normal haemoglobin-oxygen dissociation curve (normal p50) and increased sensitivity to EPO in erythroid cells in in vitro stem cell cultures are present. Over 160 mutations related to PFE have been identified. However, the genetic cause is known in approximately 70% of patients and the other patients are referred to as idiopathic erythrocytosis. In this day, a total of 8 different EPO receptor mutations have been identified [4].

As of today, OMIM classification is used in PFE classification based on genetic causes. According to this classification, some groups have a higher cardiovascular risk. One of these is the well-known Chuvas polycythemia, which was found in a region in Russia. Comparatively, this group has been the most studied group of patients with PFE and increased cardiovascular risk and thrombus have been clearly proven [5].

Hyperviscosity, which increases with the increase in red blood cells and haemotocrit in PFE patients may cause early morbidity and mortality. The Chuvash Cohort is the most comprehensive study in terms of defining clinical features.This study demonstrated decreased survival with increased arterial and venous thrombosis and haemorrhagic events in patients with Chuvash polycythemia [5].

Neutrophil-to-lymphocyte ratio (NLR) is a biomarker calculated as a simple ratio between neutrophil and lymphocyte counts in peripheral blood measurements [6]. Neutrophils are the first line of the immune response against pathogens and secretes proinflammatory and immunomodulatory cytokines and chemokines. Other cells of the immune system; dendritic cells (DC), B cells, NK cells, CD4, CD8, γδ T cells and, mesenchymal stem cells interact with neutrophils [7].

An isolated increase in the number of neutrophils with a relatively unchanged or unchanged number of lymphocytes favours neutrophils in the Neutrophil/Lymphocyte Ratio (NLR). The N/L ratio increases in bacterial or fungal infections, acute stroke, myocardial infarction, atherosclerosis, severe trauma, cancer, postoperative complications, and tissue damage that activates SIRS. In the Rotterdam study [8] NLR levels were shown to be independently and significantly associated with the risk of all-cause mortality. It has been suggested that the early increase in NLR after acute physiological stress (<6 h) may make NLR important as an earlier marker of acute stress than other laboratory parameters (e.g. white blood cell count, bacteraemia, CRP). However, although increased NLR is an independent prognostic factor of morbidity and mortality in several diseases, its normal ratio is still a matter of controversy [9]. A large retrospective case-control study [9] reported that normal NLR values in an adult, healthy non-elderly population can range from 0.78 to 3.53, while the Rotterdam study reported that the mean NLR in the general population was 1.76. It was also found that the mean NLR was significantly higher in males (mean 1.88) than in females (mean 1.68) and that individuals older than 85 years (mean NLR 2.13) had significantly higher NLR rates compared to individuals aged 45-54 years (mean NLR 1.63) [8].

Several studies have shown that NLR may be predictive of cardiovascular events [10]. In the general healthy population, an NLR > 4.5 has been suggested to predict coronary artery disease as an independent marker. Furthermore, the same study showed that using the NLR allowed the Framingham Risk Score (FRS) to accurately classify individuals in the intermediate risk category as having a lower or higher probability of cardiovascular mortality [10]. Recent evidence has shown that NLR has predictive value for disease severity and mortality across the spectrum of acute coronary syndromes, independent of the revascularisation procedure, and may be associated with other conventional risk factors. It was emphasised in the study that NLR should be accepted as 1-2 in normal population, and between 2.3- 3.0 should be considered as grey zone and especially as a warning for atherosclerosis [11].

The prognostic values of NLR have also been analysed in diabetic patients, showing that NLR independently predicts major adverse cardiac events in these patients. In a cohort of 324 elderly patients, an NLR greater than 3.68 significantly predicted atherosclerotic carotid plaque formation.[12].

Another study reported that in hospitalised hypertensive patients over 80 years of age, high NLR, especially for values > 2.97, may be an independent predictor of all-cause 3-month mortality[13].It is immunologically accepted that the reason for the increase in NLR in cardiovascular diseases is inflammation and oxidative stress in the pathophysiology of atherosclerosis and endothelial dysfunction and the disruption in homeostasis between IL-1 and its antagonists as a result of activation of the NPL3 inflammasome [14].

However, as new data on the role of non-myeloid inflammatory

cells, especially T lymphocytes and monocytes, in the immune thrombosis process have become available, it has been shown that T-reg lymphocytes are involved in the regulation of the prothrombotic effect of activated neutrophils in the process of fibrin formation and dissolution. Based on this information, the neutrophil to lymphocyte ratio (NLR) can be considered as the synthesis of these two opposing actions in thrombotic events and may play a role as a prognostic marker of cardiovascular events in PV, as shown in the general population and in a small series of patients with ET [15].

Thrombotic events are an important complication and the most common cause of mortality in patients with PV. Therefore, early assessment of the risk of occurrence and progression of thrombotic events and early intervention may be effective in improving the prognosis of patients with PV. Damage to vascular endothelial cells may induce aggregation of platelets and thrombosis as a result of increased neutrophil count and inflammation [16].

Material and Methods

Patients

We retrospectively included 50 polycythaemic patients (2 women, 48 men) who were admitted to the Haematology Outpatient Clinic of Başkent University Medical Faculty Istanbul Hospital and diagnosed as PFE. Patients with JAK2 (+) (V617F and Exon 12), malignancy with high EPO level, known lung and cardiac diseases were excluded. The age range of the patients was 18-70 years. Complete blood count was analysed in healthy periods of all patients in the absence of active infection and Neutrophil/Lymphocyte ratio and counts were determined.

Statistical evaluation

GraphPad Prism 9.5.0 statistical package programme was used for data analysis. The variables belonging to the patient group to be included in the study were given as mean and standard deviation. One-Sample t test was used to test whether the N/L ratio was different from 2.30. The correlations between two continuous variables were evaluated by Pearson correlation analysis. A value of $p<0.05$ was considered statistically significant.

Ethical Approval

This study was approved by the Ethics Committee of Baskent University Faculty of Medicine (Date: 2024-02-06, No:KA24/65).

Results

Fifty patients were included in the study, 2 of these patients were female and 48 patients were male. The mean haemoglobin of the patients was 17.7 gr/dl and the mean haemotocrit was 52.5%. The mean WBC value was 8,835 x109/L, neutrophil and lymphocyte counts were 6,035x109 /L and 1,9x109/L respectively. Notrophil/lymphocyte ratio of all patients was 2.86 (Table.1). While NLR ratio above 2.3 was considered significant, only 3 patients had NLR below this value. This value was found to be above 2.3 in all other patients. There was no statistically significant difference between the patients in the comparison of NLR within the whole group (Table 2). As seen in Table 2, it was tested whether the NLR variable of the patients was different from the value of 2.30, which is considered normal in the population, and it was found that the NLR variable was statistically significantly different from the value of 2.30 (p=0.010). Accordingly, it is possible to say that the mean of the NLR variable obtained for our sample is considerably higher (4.28) than the accepted value of 2.30 (Figure 2). As seen in Table 3, when the correlations between the variables are analysed, it can be said that there is a positive correlation between NLR and WBC ($r = 0.280$, $p = 0.049$) and PMN ($r = 0.478$, $p = 0.000$) variables and a negative correlation with the lymphocyte ($r = -0.566$, $p = 0.000$) variable.

It can be also said that; there is a positive correlation between the HB variable and HET ($r = 0.675$, $p = 0.000$), a positive correlation between the HET variable and LENF ($r = 0.352$, $p = 0.012$), a positive correlation between the WBC variable and PMN ($r = 0.935$, $p = 0. 000$) and LENF ($r = 0.516$, $p = 0.000$) variables, and there is a positive correlation between PMN variable and Lymphocyte ($r = 0.354$, $p = 0.012$) variable.

Discussion

Unlike polycythemia vera, PFE does not involve the bone marrow. Thus, there is no risk of transformation to other bone marrow malignancies such as Myelofibrosis or Leukaemia. This advantageous feature also brings some disadvantages. The most important of these is the reluctance to follow up patients in Hematology outpatient clinics due to the fact that they are not accepted as a hematological disease, and the reluctance to follow up patients in Internal Medicine and Cardiology outpatient clinics due to their polycythemia, in a manner to leave the patients hanging. The fact that they are polycythaemic makes them particularly prone to venous thromboembolism. Increased diastolic load on the heart due to increased viscosity creates a tendency to cardiovascular and cerebral thromboembolism in patients. At the same time, considering the familial transmission of the disease, it is a disease that can cause serious mortality, especially at a young age. A study has shown that a haematocrit value above 45% in females and 48% in males increases the risk of MI 3 times more than the normal population [14].

The limit of polycythaemia in PFE is not different from other polycythaemias. The fact that the disease is an interdisciplinary disease and does not belong to a branch has also reflected the number of studies in this field. The number of studies showing the cardiovascular and thromboembolic events and risk of this disease is very few in the literature and most of the studies include Polycythemia Vera patients. In one of the few studies, it was shown that high haematocrit was an independent factor for cardiovascular risk in PFE patients [17]. The treatment of the disease is intermittent phlebotomy, which has been shown to reduce complications in the literature [18].

Atherosclerosis is still one of the leading causes of mortality in the world. Therefore, NLR has recently been suggested to have an early predictive value at the very beginning of early atherocycotic events. NLR has recently been emphasised in both cardiovascular mortality in atherosclerosis and cancer studies. However, it was also used as a marker of early infection in the earlier period before CRP, especially in COVID 19 infection [19]. NLR has been used in PV patients for the same reasons, as polycythemia predisposes to atreosclerosis and early thromboembolic events. This study showed that the absolute

number of neutrophils and lymphocytes was higher (mean: 6.9 × 10^9/L, p = 0.022) and lower (mean: 1.3 × 10^9/L, p = 0.002) in the univariate analysis of NLR, respectively, resulting in an overall higher NLR. The significant finding of the study was the linear correlation between the absolute number of lymphocytes and the risk of events [20]. It was shown in the study that patients with lymphocyte counts lower than 2 × 10^9/L at baseline were at risk for venous thrombosis, and as lymphocyte counts increased, the risk for venous thrombosis gradually decreased. These changes were found to be consistent with the adverse prognostic tendency of neutrophils, indicating that the risk increases as neutrophil counts increase [21]. Although the role of lymphocytes in the underlying pathophysiological process here is unclear, lymphocytes modulate the activity and attraction of innate immune cells during clot dissolution, a special subset of T-reg lymphocytes that accumulate in venous thrombi and are vital for clot dissolution has recently been identified. These mechanistic studies have suggested an active interaction between innate and adaptive immune

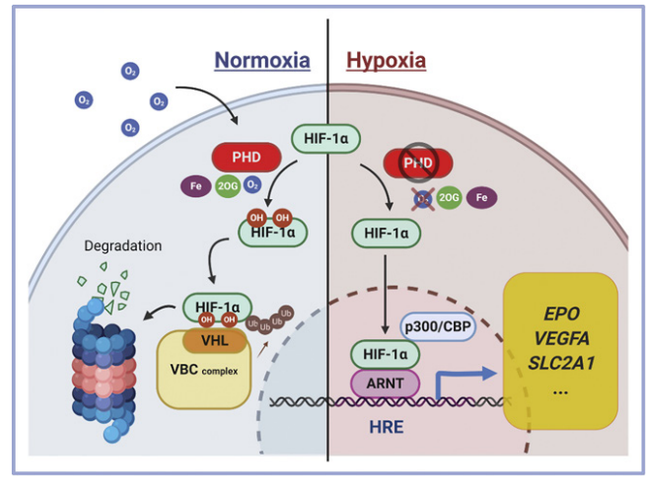

Figure 1. Hypoxia pathway (Hoffman Ronald Hematology, chapter 67)

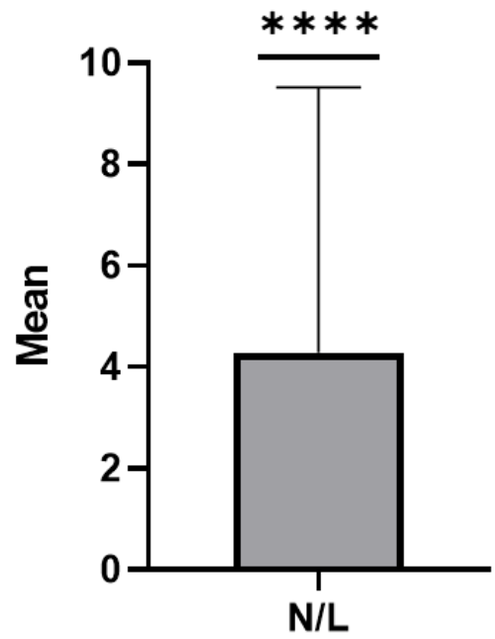

Figure 2. N/L Ratio Variable, ****p<0.000

Table 1. Descriptive statistics of patient variables

Variables	Mean ± SD	Median (IQR)
Age	51.12±14.99	50.50 (22.25
HB	22.69±26.59 gr/dl	17.70 (1.63) gr/dl
HTC	53.38±3.14	52.50 (3.30) %
WBC	9116.80±2424.17x109/L	8835.00 (3232.50) x109/L
PMN	6284.40±1989.28x109/L	6035.00 (3270.00) x109/L
LENF	1905.00±748.61x109/L	1900.00 (1140.00) x109/L
N/L	4.28±5.23	2.86 (1.80)

Table 2. Comparison of N/L Ratios of Patients According to 2.30 Value

Variable	Accepted value	Mean±SD	p*
N/L	<2.30	2.11±0.18	0.000
	>2.30	4.58±5.51	

*One Sample t Test, p<0.05 is statistically significant.

Table 3. Correlation Analysis Results Between Variables

	N/L	Age	HB	HCT	WBC	PMN	LENF
N/L	1						
Age	r = 0.092 p = 0.527	1					
HB	r = -0.172 p = 0.232	r = -0.172 p = 0.233	1				
HCT	r = -0.059 p = 0.682	r = 0.071 p = 0.623	r = 0.675** p = 0.000	1			
WBC	r = 0.280* p = 0.049	r = -0.201 p = 0.161	r = 0.021 p = 0.884	r = 0.239 p = 0.095	1		
PMN	r = 0.478** p = 0.000	r = -0.142 p = 0.325	r = -0.030 p = 0.838	r = 0.201 p = 0.161	r = 0.935** p = 0.000	1	
LENF	r = -0.566** p = 0.000	r = -0.184 p = 0.202	r = 0.188 p = 0.192	r = 0.352* p = 0.012	r = 0.516** p = 0.000	r = 0.354* p = 0.012	1

*p<0.05, **p<0.01, Pearson correlation.

systems in venous thrombosis [22]. We did not find any study similar to our study in PFE patients in the literature reviews we performed during our study. Therefore, our study is the first study investigating NLR in PFE patients.

In our study, we found a statistically significant increase in NLR with a mean of 2.8 in almost all PFE patients. Thus, we suggest that PFE patients are prone to early atherocyclosis and cardiovascular complications. Studies have not clearly demonstrated how polycythemia initiates atherosclerosis independent of hyperlipidaemia. The most important of these is diastolic dysfunction, which occurs in all polycythaemic patients with an increase in cardiac load and end diastolic pressure due to increased blood viscosity. We have demonstrated diastolic dysfunction and increased carotid intima-media thickness in PFE patients in a TÜBİTAK project in this group of patients [23]. We would like to get an answer to the question of whether NLR as a marker of atresclerosis can be helpful in the monitoring of atherosclerosis in future studies. In future studies, we think that NLR follow-up may be useful especially in patients who reach target haematocrit levels with phlebotomy. A decrease in N/LR can be expected after increased neutrophils in increased inflammation are rendered normocytemic by phlebotomies. PFE is common and the pathophysiology of the disease remains many questions. It is possible that the results of large-series studies will contribute to the world of science and increase the quality of life of patients by prolonging their survival.

Conclusion

PFE patients present with polycythaemia and in the light of limited studies, it has been accepted to cause atherosclerosis and cardiovascular events. Recently, NLR has been recognised as a very early marker of inflammation during the proinflammatory phase and has been used especially in atherosclerotic diseases. It is increasingly recognised that increased neutrophil count in the early period increases NLR and its early use may be a harbinger of atherosclerosis. In our study, we evaluated NLR which has never been performed in PFE patients before. In almost all patients, the result was above 2.3, which is considered to be the normal limit. Thus, our data suggest that PFE patients may be prone to atherosclerosis.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or compareable ethical standards.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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